helicase necessary for initiating homologous DNA recombination, and FtsK, a DNA pump involved in chromosome dimer resolution, actively disrupts nucleoprotein complexes, including RNA polymerase (RNAP) holoenzyme. RecBCD pushed and eventually displaces RNAP, Lac repressor, EcoRI(E111Q) and even nucleosomes. FtsK pushed RNAP but was able to either push or bypass EcoRI(E111Q). We conclude that RecBCD acts as a powerful stripase that overwhelms potential roadblocks. In contrast, FtsK is able to bypass some roadblocks, possibly by dissociation and reassembly ahead of the block.

3104-MiniSymp
Visualizing Transcription In Vivo at Nucleotide Resolution using Nascent Transcript Sequencing
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Elegant single molecule approaches have elucidated the mechanisms that cause RNA polymerase to pause during transcription. However, it is unclear whether these pausing events and their subsequent recovery occur within the cell as there are a large number of elongation factors that facilitate the progression of RNA polymerase (RNAP) through a chromatized genome. To explore the mechanisms of RNA polymerase elongation in vivo, we require experimental strategies that can observe transcription with the same resolution as can be obtained in vitro. Here we present an approach, native elongating transcript sequencing - NET-seq, that accomplishes this goal by exploiting the extraordinary stability of the DNA-RNA-RNAP ternary complex to capture nascent transcripts directly from live cells without crosslinking. The identity and abundance of the 3' end of purified transcripts are revealed by deep sequencing thus providing a quantitative measure of RNAP density with single nucleotide precision. Application of NET-seq in Saccharomyces cerevisiae reveals pervasive polymerase pausing and backtracking throughout the body of transcripts. Average pause density shows prominent peaks at each of the first four nucleosomes with the peak location occurring in good agreement with in vitro single molecule measurements. Thus nucleosome-induced pausing represents a major barrier to transcriptional elongation in vivo.

3105-MiniSymp
Modulation of the Translocation Properties of a Model Helicase by DNA Damage and Sequence Content within the Track
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In Bacillus subtilis, broken DNA ends are processed for repair by conversion to a 3′-ssDNA overhang terminated at a recombination hotspot (Chi) sequence. This reaction is catalysed by the AddAB helicase-nuclease that unwinds the DNA duplex and degrades the nascent single-strands in a Chi-regulated manner (Yeeles and Dillingham, 2007). Recombination hotspots regulate AddAB function by down-regulating nuclease activity on the 3′-strand beyond Chi and by preventing reannealing of nascent single strands via formation of a DNA loop (Yeeles et al., 2011). In this work, we have used Magnetic Tweezers to investigate the real-time dynamics of AddAB translocation on damaged or undamaged DNA and the effect of recombination hotspot recognition on this process. AddAB translocation traces showed a complex appearance with variable velocities between 200-400 bp/s at room temperature. DNA translocation by AddAB was slower and more prone to pausing in areas of high GC content which contained Chi sequences. Experiments using an AddAB mutant unable to recognize Chi showed no pauses but the same overall kinetic behavior along the track. On undamaged DNA, the pause duration followed a single exponential distribution with a decay time of 0.8 s. In contrast, very long stochastic pauses were observed on UV-damaged or nicked DNA substrates. Experiments to address the effect of recombination hotspot recognition on DNA translocation using bespoke Chi-containing substrates are ongoing and will also be discussed.
References
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3106-MiniSymp
The Nucleotide-Binding State of Microtubules Modulates Kinesin Processivity and Tau's Ability to Inhibit Kinesin Mediated Transport
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Tau's ability to act as a potent inhibitor of kinesin motility *in vitro* suggests it may actively participate in the regulation of axonal transport *in vivo*. However, it remains unclear how kinesin based transport could then proceed effectively in neurons, where tau is expressed at high levels. One potential explanation is that tau, a conformationally dynamic protein, has multiple modes of interaction with the microtubule, not all of which are inhibitory for kinesin motility. Thus, if tau can bind microtubules in distinct conformations or at unique binding sites that no longer inhibit kinesin, transport would proceed unhindered along the axon. Previous studies support the hypothesis that tau has at least two modes of interaction with microtubules, but the mechanisms by which tau adopts these different conformations and their functional consequences have not previously been investigated. In the present study we have used single molecule imaging techniques to demonstrate that tau inhibits kinesin motility in an isoform dependent manner on GDP microtubules stabilized with either paclitaxel or glycerol, but not GMPCPP-stabilized microtubules. Furthermore, the order of tau addition to microtubules before or after polymerization has no effect on tau's ability to modulate kinesin motility regardless of the stabilizing agent used. Finally, the processive run length of kinesin is reduced on GMPCPP microtubules relative to GDP-microtubules. These results shed new light on tau's potential role in the regulation of axonal transport, which is more complex than previously recognized.

3107-MiniSymp
Biophysical Studies Reveals the Specific Activities of Fidgetin, a Microtubule Severing AAA Enzyme
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Cell morphology, development, and differentiation rely on the spatio-temporal dynamics of microtubules. Microtubule dynamics and network remodeling are finely tuned in cells by the orchestrated activity of microtubule-associated proteins (MAPs). Reorganization of the microtubule network is performed by a novel class of MAPs called microtubule severing enzymes that are AAA+ (ATPases Associated with various cellular Activities) family of ATPases. The former member of this novel class of AAA+ enzymes is katanin p60, the catalytic subunit of katanin complex that regulates microtubule length and dynamics in cells during interphase and mitosis and targets to microtubule defects. The newest member of the severing enzyme family is fidgetin, which is involved in mammalian development. We have performed the first biophysical characterization of fidgetin in vitro. Interestingly, at a low concentration this enzyme removes tubulin dimers preferentially from the minus end of the microtubules, making microtubules appear to depolymerizing. At a higher concentration fidgetin severs microtubules. We find that fidgetin targets and severs GMPCPP microtubules better than taxol-stabilized microtubules. Further, fidgetin removes extended regions of protofilaments, in an activity we call "protofilament stripping". Our results indicate that fidgetin is a microtubule severing enzyme with new and specific biophysical abilities and targeting on microtubules.

Platform: Ligand-gated Channels

3108-Plat
Apo and $InsP_3$-Bound Crystal Structures of the Ligand-Binding Domain of an $InsP_3$ Receptor
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The ligand-binding domain (LBD) of inositol 1,4,5-trisphosphate ($InsP_3$) receptors ($InsP_3R$), which comprises the ~600 amino-terminal residues, is coupled to and thereby exerts allosteric control over the trans-membrane pore domain. Even when produced as an isolated construct, LBD binds $InsP_3$ with affinity and selectivity comparable to those of the whole $InsP_3R$ protein. The LBD sequence encodes the two β-trefoil folds, β-TF1 and β-TF2, followed by an armadillo repeat fold (ARF). A construct comprising only β-TF2 and ARF (termed $InsP_3$-binding core) binds $InsP_3$ with even higher affinity than

the entire LBD or the whole protein. Hence, β-TF1 is viewed as a suppressor of $InsP_3$ binding. Additional studies suggest that β-TF1 not only helps stabilize LBD but also couples its conformational changes to the gate of the ion pore. The crystal structures of β-TF1 alone and of β-TF2 plus ARF bound with $InsP_3$ have been solved separately. The latter structure reveals how $InsP_3$ is coordinated by various side-chains in the binding sites at the ARF - β-TF2 interface, and mutation of these side-chains weakens $InsP_3$ binding. Despite this progress, the fundamental question of how $InsP_3$ biases the gating conformation of LBD remains. To address this question, we have determined the crystal structure of the entire LBD of rat $InsP_3R1$ in both $InsP_3$-bound and -unbound conformations, revealing a triangular architecture. Comparison of the $InsP_3$-bound and -unbound conformations strongly implies that β-TF1 and ARF move as a rigid unit with respect to β-TF2. While LBD without $InsP_3$-bound may spontaneously transition between gating states, binding of $InsP_3$ between β-TF2 and ARF locks it in a state that would strongly bias the gating equilibrium toward the open state of the ion pore.

3109-Plat
Using Crosslinking and Mass Spectrometry to Study Glycine Receptor Allostery
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A wealth of high-resolution structural information regarding pentameric ligand-gated ion channels is now available, but less is known of the molecular details underlying complex allosteric mechanisms involved in channel gating and desensitization. Receptor allostery can be studied by identifying state-dependent distance constraints that may be used in molecular modeling of these receptors. Systematically generated single Cys mutations of the human α_1 glycine receptor (GlyR) expressed in insect cells were labeled with a clickable methanethiosulfonate-benzophenone crosslinker. After covalent ligation to Cys, crosslinks may then be introduced in the resting, open, or desensitized states by photoactivation. Including an alkyne tag on the crosslinker permits click chemistry addition of biotin, which allows for enrichment by avidin chromatography. Mass spectrometry (MS) fingerprinting of monomeric and higher-order GlyR bands on SDS-PAGE using ESI-QTOF MS/MS then allows for determination of the site of crosslinking. Our initial proof-of-principle studies conducted on purified GlyR have provided state-dependent information on this receptor. This approach may be broadly applicable to studies of any allosteric complex.

3110-Plat
Agonist-Induced Conformational Changes in the Ligand Binding Domain of Cyclic Nucleotide-Regulated Channels
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Cyclic nucleotide-gated (CNG) channels and hyperpolarization-activated, cyclic nucleotide-modulated (HCN) channels are opened by the direct binding of cyclic nucleotides (e.g. adenosine 3',5'-cyclic monophosphate, cAMP). The ligand binding domain is primarily formed by an eight-stranded β-roll followed by two helices (B and C helices). Following agonist binding to residues in the β-roll, the C helix is thought to initiate the opening conformational change by moving towards the binding pocket and transitioning from a less ordered coil to a stable helix. To further investigate this hypothesis, we expressed the soluble C-terminal domain of the HCN2 channel, which contains the cyclic nucleotide binding domain, and introduced cysteines into the C helix, which were labeled with the fluorophore bimane. Into the same helix, we placed pairs of histidines in helical register. Colored transition metal ions can bind to these histidines and quench the fluorophore by a FRET mechanism. The amount of quenching was used to estimate the distance between the fluorophore and the metal binding site in the presence and absence of cAMP. For all of the constructs tested, cAMP binding induced a large increase in metal affinity at the di-histidine binding sites, indicating a stabilization of the helical structure. We also observed a change in the total amount of quenching in some constructs indicating a change in distance between the fluorophore and metal binding site. These data suggest a conformational change within the C helix induced by ligand binding. When a similar di-histidine binding site was introduced into the C helix of intact CNG channels, binding of Ni^{2+} increased the efficacy of the partial agonist inosine 3',5'-cyclic monophosphate (cIMP), consistent with the hypothesis that stabilization of the secondary structure of the C helix is part of the gating conformational change.

3111-Plat
Neuropeptide Interaction with the Extracellular Domain of the Acid Sensing Ion Channel 1A
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Acidic fluctuations occur within the brain and contribute to multiple physiological and patho-physiological processes. Neurons possess specific molecules to sense and respond to these pH changes. In particular, many neurons express the acid sensing ion channels (ASICs). ASICs are ligand-gated ion channels activated by extracellular protons. ASICs are permeable to cations and depolarize neurons to mediate acid-dependent neuronal signaling. Some ASIC channels are also permeable to calcium and these channels are thought to play a particularly important role in neuronal processes. Specifically, ASIC1a subunits form channels permeable to calcium and contribute to multiple behaviors, seizure termination, pain, as well as neuronal death initiated by prolonged acidosis following inflammation and stroke. ASIC1a undergoes a process called steady-state desensitization in which slow, incremental acidification causes the channels to desensitize rather than undergo robust activation. Induction of steady-state desensitization prevents ASIC1a-mediated neuronal death. Yet, specific neuropeptides present within the brain limit steady-state desensitization and allow ASIC1a to activate following slow, incremental acidification. Two types of neuropeptides, the RFamides and dynorphin-related peptides, interact directly with the extracellular domain of ASICs to modulate channel gating. In this work, we explore the mechanism and protein domains responsible for modulation of ASC1a steady-state desensitization by endogenous neuropeptides. We determined that (1) RFamide and dynorphins alter ASIC gating in distinct, but overlapping ways; (2) dynorphins can compete with the inhibitory spider toxin venom peptide PcTx1 for interaction with the channels; and (3) the addition of RFamide and dynorphins synergizes ASIC1a modulation. We also present evidence that specific protein domains are involved in endogenous neuropeptide modulation of ASIC1a. Together, these studies further define endogenous neuropeptides as important modulators of ASIC1a activity and present data indicating that distinct protein domains control neuropeptide effects.

3112-Plat
Interdimer Contacts Paint a New Picture of Glutamate Receptor Activation
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The ionotropic glutamate receptors (iGluR) mediate the majority of the rapid signalling at excitatory synapses in the brain. The binding of glutamate and other agonist molecules to the ligand-binding domains (LBDs) of the iGluR provides the free energy for driving intra-LBD conformational transitions that open the gate of the ion channel. However, much less information is available about inter-LBD motions. We recently showed that disulfide crosslinks between kainate receptor LBD dimers inhibit receptor activation (Das et al, 2010, PNAS). Here, we used a combination of structural studies and electrophysiology to map the conformational transitions of the LBD dimers between different states of the GluA2 receptor. Interdimer disulfide trapping with exquisite functional sensitivity shows that the two subunit dimers must translate relative to each other during activation, with the center of the dimers moving towards the overall axis of the channel. The crosslink captures an intermediate state between resting and fully activated and has geometry (including reduced linker separation) that provides new insight to glutamate receptor activation.

3113-Plat
Computational Studies of the Molecular Mechanisms Responsible for Ca^{2+} Permeation and Mg^{2+} Block of NMDA Receptors
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Glutamate receptors are membrane proteins activated by the neurotransmitter glutamate that mediate fast synaptic excitation in the mammalian brain. NMDA receptors constitute a glutamate receptor subfamily specifically